STAIN TECHNOLOGY

VOLUME 10 JULY, 1935 NUMBER 3

PROGRESS IN THE STANDARDIZATION OF STAINS

THE CERTIFICATION OF STAINS (*Continued*)

A series of notes has been published in the last few numbers of this journal[1] concerning problems that have arisen in connection with the standardization of certain biological stains. The dyes discussed in the preceding articles have been brilliant cresyl blue, brilliant green, the eosins, erythrosin, basic and acid fuchsin, gentian, methyl and crystal violet, hematoxylin, and methylene blue. Each of these has presented its individual problem which has had to be attacked by its own special methods. In the notes that follow, the problems are discussed that have occurred in connection with the other dyes now on the certification basis.

NIGROSIN

Nigrosin has proved an unsatisfactory dye to standardize because it is an intentional mixture and at least one of its constituents is not definitely understood chemically. The main constituent of nigrosin is a dark violet indulin which is made to appear black by adding a small amount of a yellow dye to it. Under such circumstances it is natural that the composition of different lots of nigrosin should vary; and because of its extremely dark color it is impossible to make a spectrophotometric analysis of it to determine the nature of any particular sample.

Nigrosin is ordinarily employed in microscopic work as a background stain, especially in preparations of bacteria. About the only satisfactory method of standardizing nigrosin that has as yet been found is to test the sample in a staining procedure of this kind. If it proves to give an even, satisfactory-colored background, it is regarded as a sample that can be approved. Altho this is an entirely empirical method of standardization, it seems to have proved effective, and the stain companies apparently have no difficulty in duplicating a satisfactory sample after once learning just what is desired.

[1]Conn, H. J. Progress in the standardization of stains. The certification of stains. Stain Techn., **9**, 113–5, 1934; **10**, 3–5, 41–4, 1935.

PHLOXINE

The problems encountered in connection with phloxine have been unusual in that no difficulty was experienced in securing a satisfactory dye of this name, but it was found that phloxine had frequently masqueraded in the past under the names of other dyes.

Phloxine, as is well known, is closely related to eosin Y, but contains chlorin atoms in addition to those of bromin which are present in eosin Y. As a result, it is less yellow in color than the latter and less fluorescent, but has more intense staining properties. Shortly after the war, Dr. F. B. Mallory discovered that American samples of eosin would not give satisfactory results in his eosin methylene blue technic. When he had been given a variety of related dyes to test he discovered, however, that phloxine proved to give identical results with those which he had obtained using a pre-war imported sample labeled eosin. We have since become reasonably certain that this sample was a mislabeled lot of phloxine.

An even more striking case of mislabeling has occurred in regard to magdala red, a very expensive basic dye of the safranin group. This dye and phloxine happen to be so nearly the same shade that no difference can be detected by means of the spectrophotometer. This fact was taken advantage of by stain dealers before the war, and two grades of so-called magdala red were on the market; one was labeled "Magdalarot" and the other "Magdalarot echt." The former was actually phloxine or erythrosin and, naturally, could sell for a much lower price than the true product. We have subsequently learned that one stain company in the United States has tried to follow this practice, but it is one not to be encouraged. Phloxine should certainly be sold as phloxine and not as eosin or magdala red.

PYRONIN

There are two shades of pyronin; one known as pyronin G or Y, and the other as pyronin B. They differ only in that one is a methyl and the other an ethyl derivative, and vary only slightly in shade. Pyronin B is much the easier to manufacture because the yellowish compound is not a textile dye and the intermediate from which it is made has to be manufactured especially for this purpose.

Preliminary investigations seemed to indicate that pyronin B was entirely satisfactory for all purposes for which pyronin was called for. Recently, however, Scudder[2] has proposed a combination of the

[2]Scudder, Sara A. and Lisa, J. R. A preliminary report on a combined Gram-Pappenheim stain for formalin fixed tissues. Stain Techn., **6**, 51–2. 1931.

Pappenheim and Gram stains in which pyronin B does not seem to be satisfactory. Accordingly, at least one of the stain companies has put on the market a pyronin Y intended primarily for this technic.

ROSE BENGAL

Rose bengal is the instance of a dye which has been sold in too pure a form to be satisfactory. This dye is not very widely employed, but finds use as a stain for bacteria under certain special conditions; notably in films of soil. Certain samples submitted for certification about six years ago proved unsatisfactory in this technic, but it was subsequently learned that they could be made to work by adding a small amount of some calcium salt. This peculiarity, as shown later by Conn and Homes,[3] seems to be associated with the question as to whether or not a small proportion of the calcium salt of rose bengal is present in the dye. The unsatisfactory samples were apparently too pure representatives of the sodium salt of the dye. Upon learning this, however, no effort was made to have the manufacturers change their process, but the staining technic recommended has been changed so that a small amount of calcium chloride is added in making up the staining solution.

SAFRANIN O

Safranin O has been called for in various histological and cytological procedures, the most critical one being the Flemming triple stain in which it is employed with gentian violet and orange G. A certain amount of standardization has been effected in connection with this dye, but it must frankly be admitted that we are not yet at the bottom of the problem. Complaints still come in that certain samples on the market are unsatisfactory for this staining technic. It seems to be the case of some cytologists preferring one type of safranin and others a different one, but whether an actual chemical difference exists between the two types, we have so far been unable to determine. The problem is illusive because the staining technic is a very delicate one and it is hard to be sure of getting constant results upon repetition of the procedure or upon its employment in another laboratory. There is, moreover, so very little call for the dye that manufacturers do not often make up a new batch and data, accordingly, accumulate very slowly. In general, it can be said, however, that complaints of American safranins at present are not very serious.

[3]Conn, H. J. and Holmes, W. C. Certain factors influencing the staining properties of fluorescein derivatives. Stain Techn., **3**, 94. 1928.

THIONIN

When the imported stains were first excluded from the domestic market, there was difficulty in obtaining thionin because of its confusion with an entirely different dye known as thionin blue. Unfortunately, moreover, in one staining technic which was not infrequently employed by bacteriologists at that time thionin blue was specified when thionin had been intended. In order to avoid this confusion it was necessary for a few years to insist that users desiring to purchase thionin specify in their order "thionin (syn. Lauth's violet)". This was necessary for only a short time, however, because the manufacturers soon learned what was desired and nothing but the correctly named thionin has ever been certified as such by the Stain Commission.

TOLUIDINE BLUE O

Toluidine blue O has presented no serious problem, but in connection with it one peculiar bit of confusion has arisen which has not yet been straightened out. Toluidine blue O and azure A are almost identical in chemical composition and in shade. The former is a well known textile dye, while the latter, so far as we know, has never been intentionally prepared except as an oxidation product of methylene blue. Azure A is, accordingly, a more expensive dye than toluidine blue.

Rather to our surprise, a sample submitted by one of the stain companies a few years ago for certification as toluidine blue O proved on spectrophotometric analysis to be identical with azure A. The manufacturers, however, have checked over the method of preparation and insist that the intermediates employed could not possibly have yielded azure A. The situation is peculiar and has never been satisfactorily explained. It is still uncertain whether there was some mistake in the process of manufacture or the method of analysis employed does not correctly show the difference between azure A and toluidine blue.

Altho this problem is of extreme interest from the scientific angle, it is of no great significance from the practical standpoint. The two dyes are almost interchangeable and azure A can be substituted for toluidine blue in all the procedures known for which the latter is specified. It has not yet been definitely shown that toluidine blue can be substituted for azure A, but if a batch or two of the latter have actually been sold as toluidine blue, the practical results have not been very serious. Azure A, as mentioned above, is the more expensive of the two dyes; and, if it works in the place of toluidine blue, the purchaser should have little reason for complaint.

WRIGHT'S STAIN

Wright's stain is somewhat in a class by itself among the certified stains as it belongs to the group of compound dyes or neutral stains, compounds formed by combining an acid with a basic dye. In this particular instance the matter is still further complicated by the fact that the methylene blue, before combining with eosin, is polychromed, in other words converted partly into its oxidation products, the azures. The result is a very complex compound that is difficult to standardize.

This stain, as originally described by Wright, differs from the previously described Leishmann stain only in the method of polychroming the methylene blue before combining with eosin. Wright's method of preparation proved so much simpler than preceding methods for use in the laboratory that it has come into general use in America and the term "Wright's stain" is now quite generally applied to this type of blood stain. When commercial houses began manufacturing this product, however, Wright's method of preparation proved somewhat impractical. Accordingly, when the Stain Commission was first asked to put this stain on the certification basis it was found that the commercial product, in method of manufacture, was not Wright's stain at all. As it could not be distinguished from the stain prepared according to Wright's directions, and as the big demand in this country proved to be for something under the name of "Wright's stain", it was decided to let the manufacturers continue the practice of thus designating the product, even tho the method of manufacture was not that which Wright introduced.

Deciding what to call the stain, however, was only a small part of the problem. It soon proved that different batches varied greatly in their staining properties and also that even the best batch was not necessarily stable. Its instability showed up particularly in alcoholic solution, some samples improving and others deteriorating on standing. This, of course, has added further complications to the difficulties of testing Wright's stain. It must further be appreciated that the only way a satisfactory Wright's stain can be distinguished from an unsatisfactory sample is to show that it gives the proper staining reaction with all of the many types of leucocytes in the blood. Inasmuch as some of these types of cells are not very common in normal blood, it often means searching hundreds of fields before one can decide whether a preparation is well stained.

For all these reasons the standardization of Wright's stain always has been and still is a difficult problem. Again and again the suggestion has been made of taking this product off of the certification

basis. No such step has ever been taken, however, and users of the stain seem to feel that it is worth while to have a product that has been tested in the Commission laboratory, even tho entirely satisfactory standardization has not been effected. On this basis, the certification of Wright's stain continues, but we hope that users will always remember that in spite of the best intentions of the manufacturers and all we can do to standardize their product, an occasional sample will be found which will require some modification of the usual technic in order to obtain satisfactory results with it.[4]

STAINS RECENTLY CERTIFIED

In the table below is given a list of the batches of stain approved since the last one listed in the April number of this journal.

STAINS CERTIFIED MARCH 1, 1935 TO MAY 31, 1935*

Name of dye	Certification No. of batch	Dye Content	Objects of tests made by Commission†	Date approved
Methylene blue tablets	NA 8	——	For reductase test of milk	Mar. 6, 1935
Sudan III	NY 5	89%	As fat stain	Mar. 23, 1935
Giemsa stain	GGe 2	——	As blood stain	Apr. 2, 1935
Wright stain	CWr 9	——	As blood stain	May 8, 1935
Methyl orange	NM 5	92%	As histological counterstain	May 9, 1935
Crystal violet	NC 16	91%	As bacteriological, histological and cytological stain	May 9, 1935
Orange G	NO 5	88%	As histological and cytological counterstain	May 9, 1935

*The name of the company submitting any one of these dyes will be furnished on request.

†It is not to be inferred that these are the only uses for which each of these samples may be employed. The Commission ordinarily tests each dye for such of its common uses as seem to give the most severe check as to its staining value. Certification does not in any instance, however, imply approval for medicinal use.

[4]See Conn, H. J. and Margolena, L. A. Difficulties encountered in obtaining a satisfactory Wright stain. Stain Techn., **8**, 35. 1933.

THE CONTRIBUTIONS OF G. CARL HUBER TO MICROSCOPICAL TECHNIC

WAYNE J. ATWELL, *Department of Anatomy, University of Buffalo, Buffalo, N. Y.*

The recent passing of Dr. G. C. Huber, professor of anatomy and dean of the graduate school at the University of Michigan, brings to the minds of all his former students and associates his many contributions to microtechnic.

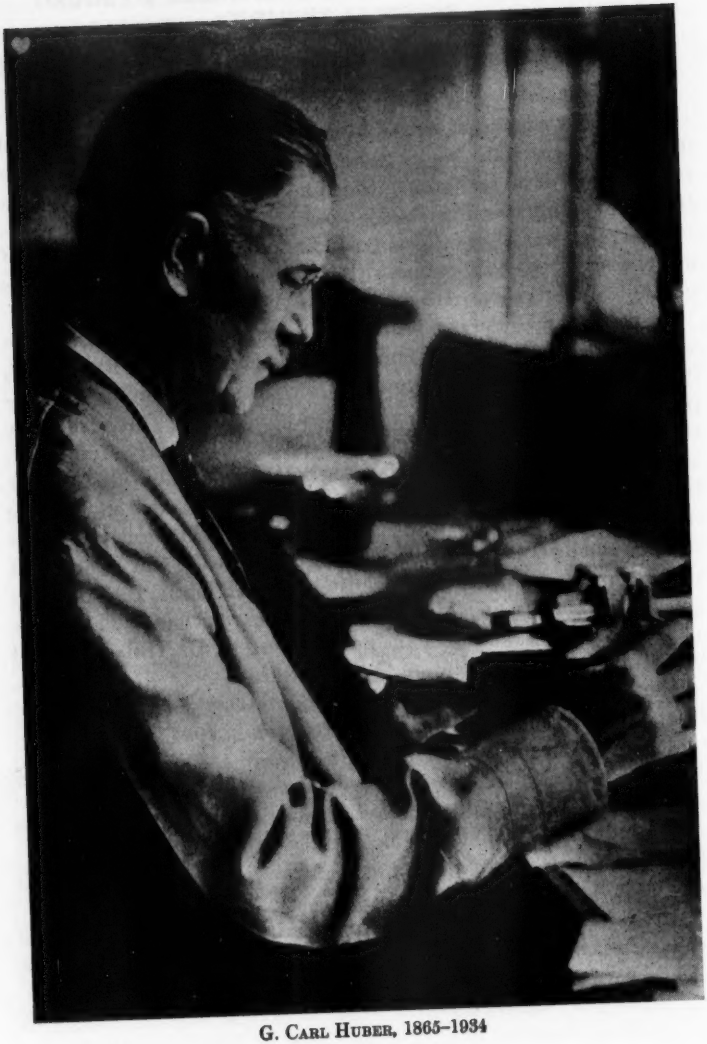
Dr. Huber was born in Hoobly, India, on Aug. 30, 1865, the son of a Swiss missionary and came with his parents to the United States at the age of 4 years. He died in Ann Arbor on Dec. 26, 1934.

Beginning his career of research and teaching at a time when microscopical technic was in its childhood, if not strictly in its infancy, it was inevitable that the young Huber should be profoundly impressed by the importance of histological methods. Interest in the structure of the nervous system, which had been greatly stimulated, scarcely more than a decade before, by the first publication of the Golgi method, was at a high point. On every hand workers were searching for new procedures by which some further detail of intimate structure might be revealed. It was during this period that Cajal, Ehrlich, Kölliker, von Lenhossèk, Dogiel, Bethe, Bielschowsky and others made many of their notable contributions. Thus early attracted to the structure of the nervous system, Dr. Huber retained this as one of his major interests thruout life.

The editing and expanding of the Böhm-Davidoff Text-book of Histology in 1900, with an extensive revision in 1904, was an opportunity for the young histologist to present, for the first time in English, many of the methods newly developed by European investigators. To these he added many methods and modifications of his own devising. It would be difficult indeed fully to evaluate the influence of this work on the subsequent development of histological technic in America.

More specifically, Dr. Huber's major contributions to microscopical technic may be considered under three heads: neurological, renal and general.

Neurological. Ehrlich's intra vitam methylene blue method seems to have been used first in the United States by Dr. Huber. Altho this cannot be substantiated by a reference to the literature, a number of colleagues, to whom this statement has been submitted, concur in believing that it is probably true.



G. Carl Huber, 1865–1934

Having long been an exponent of the vascular injection method of fixation as insuring uniform penetration and a minimum of shrinkage, he modified (1913) the Ranson pyridine-silver method by injecting the fixative and by providing for decalcification with nitric acid. By these means he was able to prepare sections of large objects such as the entire head of a laboratory animal.

Later he devised and published (1927) a method of fixation and staining of the central nervous system for the study of its cytoarchitecture. The fixative is a mixture of alcohol, trichloracetic and mercuric chloride.

Renal. During his studies on the arteriolae rectae he perfected (1907) a method of injecting the finer arterioles, which could be followed by corrosion. This was a modification of a method used by Krassuskaja. Later, in seeking to isolate the renal tubule by teasing (1911), he modified and improved the acid maceration technic employed by Karl Peter.

General. It was his custom to give to each student in his histology courses a large number of sections, stained and ready for mounting. In order to handle this great number of sections readily and to combine the advantages of sectioning in paraffin with staining in celloidin, he devised the celloidin sheeting method (1906).

Dr. Huber's long use of the wax-plate method of reconstruction made him acutely conscious of the distortion often resulting from the cutting of the sections. In order to reduce this distortion to a minimum he devised his water-on-the-knife method (1901). In this method paraffin sections are cut on a sliding microtome, the knife being kept well covered with water. As the section is cut it floats on the surface of the water and is prevented from wrinkling or curling by surface tension. In this connection it is no secret that he held the rotary microtome in slight regard for careful work. The accompanying photograph shows him seated at what was perhaps his favorite instrument, a small Altmann pattern sliding microtome which he brought home with him from one of his early trips to Germany.

He developed an injection apparatus with a pressure tank and valves using the city water supply as the pressure source (1907).

During the World War he served as Contract Surgeon and directed his attention to the devising of practical methods for the repair of peripheral nerve injuries. Among the men assigned to work with him at different times during this period were Drs. Dean Lewis, J. F. Corbett, Byron Stookey and T. Roberg. With the help of these surgeons he worked out and published the cable auto-transplant method of nerve repair (1919), the injection of absolute alcohol for

the prevention of neuromas (1920a) and the use of liquid petrolatum for the storage of nerve transplants (1920b).

It was, however, in the carrying out of established technical procedures, rather than in the devising of new methods, that Dr. Huber showed himself the outstanding master of microscopical technic that he was. He had his own little modifications for nearly every method he employed and usually they were improvements on the original. In fact, it was no unusual thing for him to secure more beautiful results with a method than the originator himself. Unsparing of time or of pains he persisted until the result was to his liking. Appreciating full well, as he has been heard to express it, "the innate cussedness of inanimate things", he still was able to enlist, to an unusual degree, the aid of the subtle forces of temperature, surface tension and the like, which often seem to array themselves to baffle the less skillful. He thus was able to work with the joy of the artist who employs a friendly medium.

As a monument to the mature skill and unflagging industry of his later years stands the splendid collection of serial sections of vertebrate brains at the University of Michigan.

REFERENCES

Böhm-Davidoff-Huber. 1900. Text-book of Histology. Saunders, Phila.

Huber, G. Carl. 1901. Studies on the neuroglia. Am. J. Anat., **1**, 45–61.

————. 1906. On a rapid method of preparing large numbers of sections. Zts. Wiss. Mikr. **23**, 187–96.

————. 1907. The arteriolae rectae of the mammalian kidney. Am. J. Anat., **6**, 391–406.

————. 1911. A method for isolating the renal tubules of mammalia. Anat. Rec., **5**, 187–94.

———— and S. R. Guild. 1913. Observations on the peripheral distribution of the nervus terminalis in mammalia. Anat. Rec., **7**, 253–72.

————. 1919. Transplantation of peripheral nerves. Arch. Neurol. and Psych., **2**, 466–76.

———— and Dean Lewis. 1920a. Prevention of neuromas by the injection of alcohol into living nerves. Arch. Surg., **1**, 85–113.

————. 1920b. Repair of peripheral nerve injuries. Surg. Gyn. and Obst., **30**, 464–71.

————. 1927. New method of fixation and staining of the central nervous system for the purpose of study of cytoarchitecture. Contrib. to Med. Sc., Warthin Ann. Vol., 1–12. Wahr, Ann Arbor.

A RAPID COMBINED FIXING AND STAINING METHOD FOR PLANT CHROMOSOME COUNTS[1]

ELSA BACKMAN, *Department of Botany, University of Michigan, Ann Arbor, Mich.*

ABSTRACT.—**A new method for rapidly preparing slides suitable for chromosome counts by the use of a combined fixing and staining solution involves the substitution of anthraquinone for picric acid in Bouin's formula and the addition of alizarin red S with a metallic salt as a mordant. The fixed smears, after being dehydrated to 95% alcohol, are differentiated in 0.5% sulfuric acid in 95% alcohol saturated with picric acid, washed, cleared and mounted in xylol-balsam. Cymene may be used to intensify the stain. Root tips fixed in the above solution may be dehydrated in dioxan, a paraffin solvent; infiltrated, embedded, sectioned and mounted in the usual way. The sections are subsequently differentiated in picro-sulfuric acid alcohol and cymene. An alternative method of differentiation for this stain is also described.**

The smear methods for plant cytology introduced by Belling and Taylor, have made possible rapid chromosome counts in pollen mother cells. This paper is the report of an attempt to secure quickly prepared permanent smears with a combined fixing and staining solution, and slides suitable for somatic counts by the use of the same solution in connection with dioxan, a dehydrating agent and paraffin solvent, recently used by Graupner and Weissberger.[2]

The substitution for picric acid in the Bouin formula of anthraquinone, a superior killing agent, makes possible the addition of a stain, alizarin red S, which has been used by Benda for staining chromosomes in sectioned material. The following formula and schedule have secured successful smears of *Gasteria* and also of several genera of Cacti, in spite of the small chromosomes and quite dense cytoplasm of the latter.

Anthraquinone, saturated solution	75	cc.
Formaldehyde	25	cc.
Acetic acid, C.P. 99.5%	5	cc.
Alizarin red S[3]	0.125	g.
Saponin	0.150	g.

[1]Paper No. 531 from the Department of Botany and Herbarium of the University of Michigan.

[2]Graupner, Heinz and Weissberger, Arnold. The use of dioxan in the embedding of microscopic objects. Zool. Anz., **96**, 204–6. 1931.

[3]A Coleman and Bell sample used in present work.

Immediately before use 8 to 12 drops of a normal solution of some metallic salt are added to each 100 cc. of this fixing solution to form a brilliant metallic lake in the chromosome. Aluminum chloride, hydrated copper nitrate, and ferric chloride have been most frequently used in this study. The concentration of the metal is important. The suitable amount of any new salt solution to be used may be ascertained by adding to a given volume of the fixing solution as many drops as it will hold without precipitation of the hydroxide during the maximum fixing time, that is, three to four hours.

1. Make the smear according to Taylor's technic.[4]
2. Fix 3 to 5 minutes, depending upon the density of the cytoplasm, in the anthraquinone-alizarin solution.
3. Wash in tap water 2 to 5 minutes.
4. Dehydrate thru the alcohol series, 2½, 5, 10, 20, 30, 50, 70, 85, and 95%, for one minute each.
5. Differentiate by flooding the slide with 0.5% sulfuric acid in 95% alcohol saturated with picric acid until the chromosomes are definite, a few seconds usually being sufficient. In some species, the cytoplasm will become almost colorless. This is not necessary, however, since the chromosomes will assume a more brilliant shade than the cytoplasm. Too prolonged an acid treatment is not advisable. If differentiation is difficult, fixation time must be shortened.
6. Wash the slide with 95% alcohol to remove the free acids.
7. If the slide is to be used immediately, intensify the chromatin stain by washing one half to one minute in 95% alcohol containing 4% by volume of cymene. The effect of this reagent on the permanence of the stain has not yet been tested.
8. Wash with absolute alcohol.
9. Clearing is done by mixtures of absolute alcohol and xylol: 5 parts alcohol, 3 parts xylol, for two minutes; 3 parts alcohol, 5 parts xylol, for two minutes.
10. Wash in xylol 3 to 5 minutes, mount in xylol-balsam.

An alternative method of differentiation, that is, flooding the slide with 0.5% sulfuric acid in 95% alcohol, washing out the free acid with 95% alcohol, neutralizing in 0.2% potassium hydroxide in 95% alcohol and intensifying the stain with the cymene solution, has also been found satisfactory.

The long series of dehydration and clearing steps given here is not peculiar to this technic, but is necessary to any good permanent smear preparation because of the short duration of the fixing period. Clove

[4]Taylor, W. R. The smear method for plant cytology. Bot. Gaz., 78, 236–8. 1924.

oil has been avoided, not only because of the shrinkage which may be caused in smears by passing directly from it to xylol, but also because it tends to obscure differentiation in this method.

The alizarin-anthraquinone solution has been tried with considerable success on root tips in the monocotyledonous genera: *Scilla, Gasteria, Aloë, Allium, Ricardia, Secale, Hordeum, Yucca* and *Agave;* and the dicotyledonous genera: *Primula, Phaseolus, Nicotiana, Muehlenbeckia,* and *Pelargonium;* using the following schedule, which requires at most eight hours:—

1. Root tips the size of those of *Allium* should be fixed three to four hours; larger ones should be trimmed. Small ones such as those of *Secale* will stain in two hours, those of *Phleum, Oenothera* and *Datura* in one to two hours. A longer time tends to make differentiation difficult. If it is not going to be possible to remove the material from the fixing bath after a short interval, the metallic salt should be omitted. Staining will then require six hours or longer. Tips have been left in a solution containing no metal for ten hours without ensuing difficulties in differentiation. The chromosomes, however, may not be as clearly defined as in those tips which have been fixed using the metal.

2. The fixed material, washed ½ hour in tap water to set the stain, is placed directly into dioxan, three changes for a total of 2 to 2½ hours. Shrinkage, should it occur in this step, can be eliminated by adding a little water to the first dioxan wash.

3. To secure infiltration, the roots are first placed in a mixture of one third dioxan and two thirds soft paraffin in the embedding oven for ½ hour; then into soft paraffin, M.P. about 46°, ½ hour; a mixture of hard and soft paraffin, ½ hour; hard paraffin, M.P. about 55°, ½ hour. Excessive heat should be avoided.

4. The material is embedded, the sections cut and mounted in the usual way and after drying the slides are placed in xylol to remove the paraffin, then washed with absolute and 95% alcohol.

5. Differentiate, wash, clear and mount according to steps 5, 6, 7, 8, 9 and 10 of the smear technic given above.

To facilitate the differentiation of cells containing very dense cytoplasm, various modifications of the fixing solution have been tried. The addition of 1 cc. of 1% calcium acetate solution to the original formula tends to darken the chromosomes. The addition of a colloid, pepsin for instance, seems to aid in clearing the cytoplasm.

For a single slide, differentiation of alizarin requires from one to three minutes, as compared to Newton's crystal violet, which requires ten to fifteen minutes. One hundred slides, differentiated ac-

cording to this method, required two hours. One hundred control slides, stained and differentiated according to Newton, required six hours. Fewer containers and bottles of reagents are needed.

The permanence of the stains produced by this method has not yet been fully tested. The slides improve for a week or more, and after four months show no indication of deterioration other than a metallic luster in the chromonemata. The use of color screens is an advantage; two gelatin-coated plates, one dyed with fast green, the other with picric acid (or one plate dyed in malachite green), have given the best effects.

The manner of handling root tips in dioxan requires a word of explanation. Since dioxan sinks below the paraffin, it is important in changing the paraffin bath that the vial be shaken before pouring off the paraffin to remove the dioxan layer, insuring the penetration of the hard paraffin. In dehydrating, enough dioxan must be used to remove all the water. The used dioxan, kept over calcium chloride, will do for at least the first change. Fresh, thoroly dehydrated dioxan must be used for the third change. Constrictions found on the chromosomes of *Scilla* root tips, prepared according to this technic, gave evidence that dehydration with dioxan cannot cause undue shrinkage or distortion. The images, however, are not as clear as in those tips which have been dehydrated in alcohol.

For storage purposes, fixed material has been left in dioxan (after three changes) for a week without noticeable difference in the stain. The chromosomes in smears which have been stored in 70% alcohol for a week can still be differentiated, not as readily nor as brilliantly, however, as those in freshly prepared smears.

Anthraquinone, a killing agent superior to picric acid, substituted for it in Bouin's formula, produces a fixing fluid for smears that is comparable to Sax's modification of Nawashin's fluid, using as a criterion the preservation of spirals in the metaphase chromosomes of *Gasteria* pollen mother cells. Parabenzo quinone, used in the Bouin formula, is also a good killing agent but much more expensive. A study is being made of the action of the more complex quinones. Experiments indicate that those containing polar groups are least effective. Thus alizarin alone, while a quinone, is a poorer killing agent. Some smears of *Gasteria* fixed in the anthraquinone-alizarin mixture indicate a possibility of use for details of chromosome structure and will be studied further.

CHLORATE-OSMIC-FORMALIN METHOD FOR STAINING DEGENERATING MYELIN

R. L. Swank and H. A. Davenport, *Department of Anatomy*,[1] *Northwestern University Medical School, Chicago, Ill.*

Abstract.—**A former chance observation that formalin added to a chlorate-osmic staining mixture appeared to stabilize and enhance the differential staining of degenerating myelin was investigated. An arbitrary formula was developed: 1% $KClO_3$, 60 cc.; 1% OsO_4, 20 cc.; glacial acetic acid, 1 cc.; and formalin, 12 cc. (all aqueous solutions) which gave clear backgrounds and well stained degeneration. Brain and spinal cord were stained in this 7 to 10 days after formalin fixation for 2 days. The possible relationship of a staining mixture buffered with regard to oxidation-reduction and its staining effects is discussed.**

Former investigations of the Marchi stain (Swank and Davenport, 1934a, b, 1935) have shown that light backgrounds with well stained degeneration were obtainable, but in all but a few instances staining artefacts (referred to as interstitial granular deposits or dust) have so obscured the field that fine and scattered degeneration could be identified only with difficulty.

Believing that the method was basically at fault, the writers reinvestigated a former lead (1934a), namely the $KClO_3$-osmic acid stain to which formalin had been added. A former difficulty (diffuse staining of the center of the block) has been partly overcome by an increase in the percentage of $KClO_3$ and the addition of formalin to the stain. The following procedure has proved much more reliable than older methods and has been successful in the hands of three independent workers in two different laboratories.

1. Degeneration time of approximately 14 to 20 days has been found to be optimum for cats; 8 to 35 days have given very good results. For rabbits 10 days seems to be near optimum.

2. The animal is killed by an overdose of nembutal or some other barbiturate intraperitoneally. *Ether should not be used* (Swank and Davenport, 1935; Weil, 1929).

3. The animal may be perfused with 2.5 to 5% anhydrous (or 5 to 10% crystalline) $MgSO_4$ solution combined with 2 to 3% $K_2Cr_2O_7$. The left ventricle is opened and a cannula is inserted and tied into the aorta. The vena cavae are opened to allow free drainage of venous blood. *Physiological saline solutions, formalin, or Mueller's fluid alone should not be used.*

[1]Contribution No. 215.

4. Immediately after perfusion, the brain and spinal cord are removed and put into 10% formalin for 48 hours.

5. The tissues to be stained are cut into sections 3 mm. thick and placed directly, *without washing*, into the following staining solution (formula for 93 cc. of the stain):

1% aqueous solution of $KClO_3$	60 cc.
1% aqueous solution of osmic acid	20 cc.
Glacial acetic acid	1 cc.
37% formaldehyde gas solution (Merck's reagent used)	12 cc.

Approximately 15 volumes of stain to 1 volume of tissue is sufficient. *During staining, the solution should be agitated daily and the pieces of tissue turned over.*

6. After staining for 7 to 10 days, tissues are washed in running water for 12 to 24 hours; dehydrated in 70%, 95%, and absolute alcohols; put into ether-alcohol; and embedded in celloidin or low viscosity nitrocellulose (Swank and Davenport, 1934c). Blocks are cut and the sections mounted in xylene-balsam with glass cover slips.

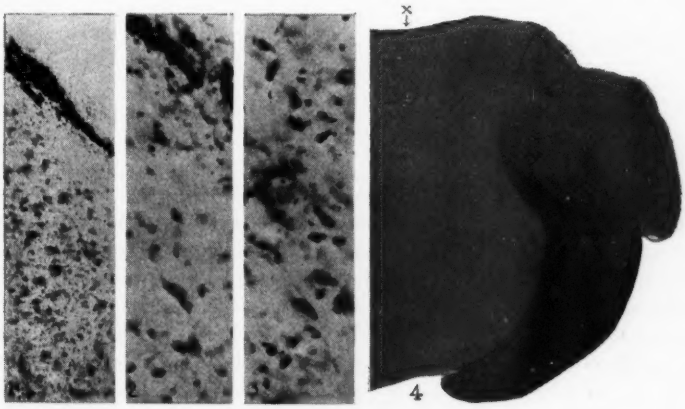
If desired, counterstaining with cresyl violet can be done in the following manner. After cutting, the sections are placed directly in a 1% aqueous solution of the stain for 3 to 4 minutes. They are then washed (2 sec.) in 70% alcohol and differentiated in acid alcohol (1 part HCl to 10,000 parts of 95% alcohol) or in a solution of equal parts of 95% ethyl alcohol and butyl alcohol. Sections should be thoroly washed in several changes of 95% ethyl alcohol, then butyl alcohol, and mounted in Canada balsam as before. The appearance of a counterstained preparation is shown in Figs. 1 to 4.

An alternate fixing method is the removal of tissues *without perfusion* and their fixation directly in 10% formalin for 48 hours. This has given very good results and takes less time. An alternate perfusion fluid is Mueller's fluid + 5% $CuSO_4 \cdot 5H_2O$ (solution must be filtered before using). This acts as a mordant for counterstaining with cresyl violet.

The high percentage of potassium chlorate (0.6%) and acetic acid (1%) prevents staining completely if formalin is omitted from the staining solution (Swank and Davenport, 1934a). Apparently the formalin acts somewhat specifically upon the degenerating myelin, as interstitial granular deposits (Swank and Davenport, 1935) are almost completely absent. By increasing the concentration of the acetic acid or decreasing the formalin in the solution, staining can be inhibited and by increasing the formalin it can be enhanced.

The density of staining can be controlled also by varying the concentration of the $MgSO_4$ in the perfusing solution. The higher concentration (10% of the hydrated or 5% anhydrous) increases the

tendency of degenerating myelin to stain and will occasionally cause a few normal myelin rings to be colored black. Reducing the $MgSO_4$ to half this concentration or doing away with perfusion entirely will result in a lessened tendency to stain. Probably it is better to have a few normal myelin rings stained than to have the inhibition sufficient to prevent their staining. There seems to be less likelihood of inhibition of staining of degenerating fibers under such conditions, and no difficulty should be encountered in differentiating stained normal from degenerating fibers. Hypertonic $MgSO_4$ solution shrinks the brain, facilitates its removal, and reduces the presence of artefacts (interstitial granular deposits) to a minimum (Swank and Davenport, 1934b, 1935). For emphasis, let us restate that perfusion with NaCl solutions causes an increase in the interstitial granular deposits (Swank and Davenport, 1935).



EXPLANATION OF FIGURES

1. Photomicrograph of an area taken from point *x* of Figure 4 stained by the chlorate-osmic-formalin method and counterstained by cresyl violet (× 90).
2. Photomicrograph of an area taken from point *x* of Figure 4 stained by the chlorate-osmic-formalin method and counterstained by cresyl violet (× 360).
3. Photomicrograph of an area taken from point *y* of Figure 4 stained by the chlorate-osmic-formalin method and counterstained by cresyl violet (× 360).
4. Photomicrograph of section thru the mesencephalon of a cat following right retinectomy stained by the chlorate-osmic-formalin method and counterstained by cresyl violet (× 11). Note the degenerating optic tract entering the lateral geniculate body and the thin group of degenerating fibers along the dorsal surface of the section at *x*.

Altho the chlorate-osmic-formalin solution would seem to be an incompatible mixture, it is stable and appears to possess the properties of a buffer with respect to an oxidation-reduction system. The conclusion that it has such buffer action has been drawn from observations that tissues stained by it seem to stain more uniformly than

by mixtures not a combination of oxidizing and reducing agents. Also, the quantitative composition of the mixture is less critical than chlorate-osmic mixture without formalin.

The potentials of the various staining solutions were tested with a Leeds and Northrup potentiometer. A platinum wire electrode was used and the calomel half-cell was reversed in order to obtain readings. It was found that solutions which when added to the stain

TABLE 1—POTENTIAL IN VOLTS OF STAINING SOLUTIONS. PLATINUM WIRE ELECTRODE; CALOMEL HALF-CELL (SATURATED KCl) REVERSED IN THE CIRCUIT

Composition of the solution	Potential
1. Marchi's solution	0.41
2. Marchi's solution + 2% acetic acid	.48
3. 1% $KClO_3$, 1% osmic acid and glacial acetic in the proportions: 60–20–1	.58
4. Solution 3 + 6 parts (in 87) formalin	.44
5. " 3 + 12 " (in 93) "	.41
6. " 3 + 24 " (in 105) "	.37
7. " 5 without acetic acid	.30
8. " 5 with 2 parts (in 94) acetic acid	.44
9. " 5 " 5 " (in 97) " "	.44
10. " 5 after staining tissue for 7 days	.51

inhibited staining (acetic acid, potassium dichromate, and potassium chlorate) increased the potential, while solutions which enhanced staining (formalin and ammonium hydroxide) decreased the potential. The chlorate-osmic solution *without formalin* has a relatively high potential, but addition of formalin reduces it to approximately the same level as that possessed by the Marchi stain (Table 1). At the end of seven days during which tissues were being stained, the potential of the chlorate-osmic-formalin mixture rose to the same level possessed by the stain without formalin, altho the odor of osmic acid was still quite strong. This suggested that an addition of formalin to the stain after several days staining, to reduce the potential, might increase the penetration as well as effect economy. This step has been tried in two blocks without harm being done, but needs further investigation to determine its effect on penetration.

REFERENCES

SWANK, R. L., and DAVENPORT, H. A. 1934a. Marchi's staining method: Studies of some of the underlying mechanisms involved. Stain Techn., **9**, 11.

————. 1934b. Marchi's staining method: II. Fixation. Stain Techn., **9**, 129.

————. 1934c. Embedding with low viscosity nitrocellulose. Stain Tech., **9**, 137.

————. 1935. Marchi's staining method: III. Artefacts and effects of perfusion. Stain Techn., **10**, 45.

WEIL, ARTHUR. 1929. Veränderungen in dem histologischem Bilde und dem Lipoidaufbau des Zentralnervensystems in der Äthernarkose. Archiv. Gesamte Physiol., **223**, 351.

SUGGESTIONS ON THE USE OF THE MICROSCOPE

S. I. Kornhauser, *Dept. of Anatomy, School of Medicine, University of Louisville, Louisville, Ky.*

Undoubtedly many microscopists modify their stock instruments to fit their own ideas of usefulness, comfort, and convenience. It is hoped the following experiences of the author may prove useful to others.

1. Low Fine Adjustment. With slanting binocular eyepieces the microscope may comfortably be raised above the level of the table. On my microscope board (Figs. 2 and 3) the distance is 2½ inches. A low fine adjustment in such a case is very much less tiring and is handy in shifting to the iris-lever beneath the substage condenser. Altho only newer stands are provided with low fine adjustment, inexpensive substitutes such as the one described can be added when necessary. The one shown in the figures consists of a wooden cylinder, a U-shaped piece of iron, a stove bolt which is the axis for the cylinder, and a screw to hold the apparatus to the base of the microscope. The whole was constructed by the writer in a few hours and has proved very efficient. (Fig. 1.) A short piece of inch dowel stick was drilled lengthwise along its central axis to fit the ⅛" stove bolt with-

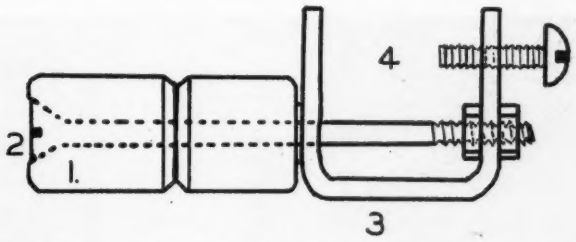


Fig. 1. Low fine-adjustment attachment seen from above. × 1. 1. Wooden cylinder. 2. Stove bolt secured at right with two nuts. 3. U-shaped iron. 4. Screw for holding attachment to the stand. Between 1 and 3 is a thin copper washer.

out binding. It was then turned down on a wood lathe to make a knob which fitted the hand comfortably, a groove for the belt, and a shoulder to extend to the left arm of the iron U. The diameter of the cylinder at the belt groove was made equal to that of the shoulder on the brass fine adjustment knob of the microscope so that both had the same speed of rotation. Two holes ⅛" in diameter, in line and parallel to the base of the U, were drilled to take the stove bolt. The

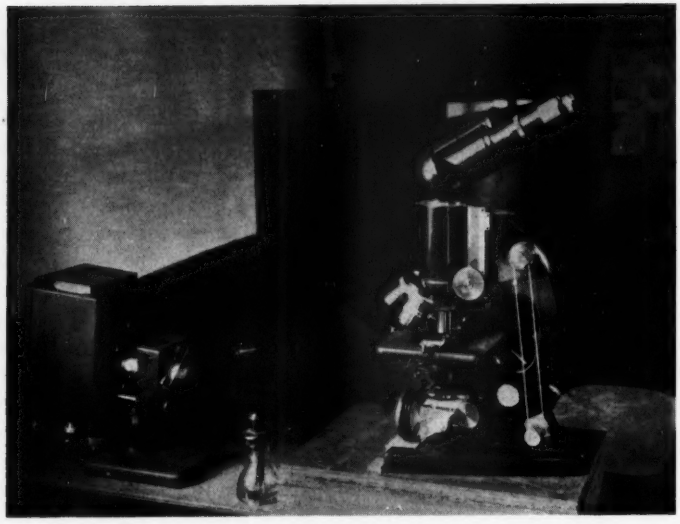


Fig. 2. View of microscope, accessories, and table from the left.

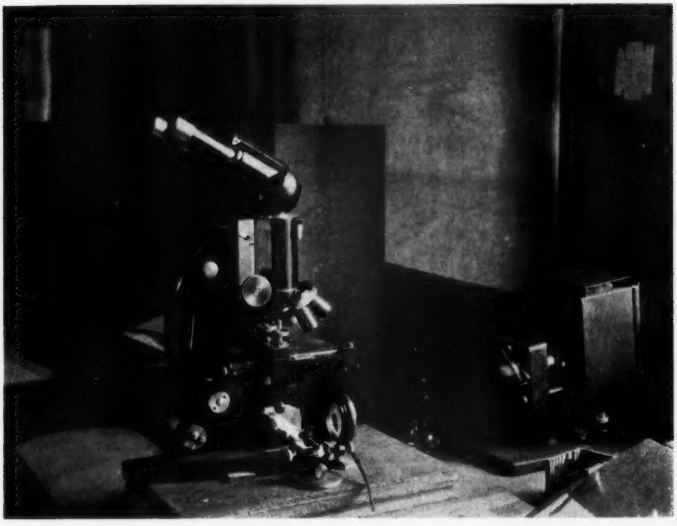


Fig. 3. Same from the right.

wooden cylinder was slipped onto the bolt which was inserted thru the two holes in the U from the left and was fastened to the right arm of the U by two nuts, one inside and one outside. (Fig. 1.) Also on the right of the U is a threaded hole with a machine screw to attach the apparatus to the base. Since I use the left hand principally for the fine adjustment and iris, and the right for the other manipulations, the supplementary fine adjustment was made only for the left side, but with little modification it could be made for both sides. The belt is a piece of string rubbed with dry resin.

2. Ninety Degree Prism Instead of a Mirror. A right-angled prism of sufficient size to fill the condenser aperture was fastened to the plane side of the stock mirror with two strips of Dennison's Scotch tape. Between the prism and the mirror was placed a piece of blotting paper blackened with India ink. This avoided a second reflecting surface and gave a single clean-cut image of the lamp filament on the iris of the substage. The combination I now use, the ribbon filament lamp, the prism, and the Bausch & Lomb achromatic condenser is most excellent. When the condenser is immersed one can use an oil immersion objective of N.A. 1.40 to good advantage. The prism measured 1½ inches × 1½ inches on its right angle faces. It was the upper prism of a U. S. Army Officer's trench periscope made by Wollensock. Several of these periscopes were purchased from Jim Croft, Davenport, Iowa, for a few dollars each.

3. Supplementary Condenser for Objectives of 20× or Less. With a condenser of high numerical aperture, the field of the lower powers is seldom evenly illuminated unless one is continually racking the condenser up and down. This is a great nuisance if one is using the condenser immersed for critical work. Thus the advantage of the supplementary condenser (now supplied by various manufacturers) to swing in and out of the optical axis beneath the iris of the substage. The writer has constructed a supplementary condenser which is used between the light source and the prism described in item 2. In Figure 1 the condenser is shown in place. In Figure 2 it is swung to the right. It is only used for objectives of 20 magnifications or less. This condenser consists of a biconvex lens 45 mm. in diameter and having a focal length of 110 mm. It is fastened with Ambroid cement to part of a discarded mounting of a swing-out substage condenser. This in turn is clamped to the microscope board (Fig. 2). A lever is attached to the mounting to swing the condenser in or out of the path of rays. The correct position of the condenser may be obtained by removing the eyepiece and view-

ing the back lens of the low power objectives when they are in focus. A clear, evenly and fully illuminated objective should result. A cork of the proper height makes a good rest for the condenser when in use. The lens for this condenser was one of the elements of the objective of an old Victor projecting lantern.

4. Extension for Lever on Substage Iris. On most microscopes this lever is too short or ends in a ball. A convenient extension, one easily found and moved without groping, may be constructed out of a piece of thin brass tubing 4 mm. in diameter and about 40 mm. long. This can be easily attached to the stock lever (slipped over in many cases). Such a lever is seen in Figure 2 directly above the prism.

5. Sponge Rubber Pads. In long sessions at the microscope, the forearms resting on the edge of the table get chaffed. To obviate this difficulty sponge rubber kneeling pads (such as may be purchased at any five and ten cent store) 13 inches $\times$ 6½ inches are tacked to the table, to right and left of the microscope board (Figs. 2 and 3). They are allowed to project about 2 inches beyond the edge of the table so as to fold down when the arms come in contact with them. These are a real comfort.

6. Dust-proof Covers for the Microscope. Glass covers and cellulose-acetate covers are expensive and not too efficient. How often one sees the sun pouring thru a glass bell jar on a microscope! Good, durable and inexpensive covers can be easily constructed out of Masonite, a hard, smooth presdwood which can be obtained in sheets ⅛″ thick from most lumber firms. This material is easily sawed. The pieces are then firmly held together by strips of quarter round, along the inner angles, glued from the inside and held with small nails from the outside. A wooden knob on the top completes the cover. Such a cover is seen in the background in Figures 2 and 3 (directly behind the microscope). Similar covers are also excellent for microtomes and other laboratory instruments. The material for a cover such as shown in the photograph costs not over fifty cents.

7. Study Board. In taking notes about slides and making sketches, pads of paper 3 $\times$ 5 inches are used. These may be filed for reference. To use these conveniently and to avoid the continual use of thumb tacks, a piece of Masonite 8 $\times$ 12 inches inclined about 30° is drilled to take two regular microscope stage clips (Fig. 3, lower right). These clips hold a single sheet firmly and the Masonite makes a nice drawing surface. The ease of changing sheets and absence of wear and tear on one's finger nails may appeal to others.

A NEW FIXING FLUID AND A REVISED SCHEDULE FOR THE PARAFFIN METHOD IN PLANT CYTOLOGY

L. F. RANDOLPH, *Department of Botany, N. Y. State College of Agriculture and Division of Cereal Crops and Diseases, U. S. Dept. of Agric., Ithaca, N. Y.*

An improved chromo-acetic-formalin fixing fluid, designated by the abbreviation "Craf" was developed several years ago primarily for the purpose of making chromosome studies in *Zea*, and since that time has been used extensively with very favorable results. The new Craf fluid was compared with the fluids of Allen, Benda, Delaunay, Flemming, La Cour, Levitsky, and Navashin in the preparation of root tips for chromosome studies, and it gave consistently better results than were produced with these fluids; the outline of the individual chromosomes is more distinct, there is less shrinkage, and the spindle insertions and other morphological features, as well as the metaphase doubleness of the chromosomes, are sharply delimited. With the Craf formula a very satisfactory fixation of the somatic chromosomes was obtained in a variety of plants other than maize, including *Crepis*, *Hordeum*, *Iris*, *Poa*, *Secale*, *Solanum*, and *Triticum*. The chromosomes in smear preparations of the sporocytes of *Tradescantia* and other plants also are well preserved with this method. After fixation with Craf, the formed chromosomes stain readily with crystal violet, gentian violet and iron-alum hematoxylin, and produce a strong contrast with the cytoplasm, which ordinarily does not retain the stain.[1]

The schedule for the paraffin method in common use among cytotechnicians is long and tedious. Tests which were conducted in an attempt to simplify the procedure and at the same time to bring about as little alteration as possible in the material resulted in the development of the following schedule. It was discovered that the customary washing in water and dehydration in the lower alcohols could be dispensed with after fixation in Craf, and the schedule was shortened still further by using normal butyl alcohol in place of ethyl alcohol and xylene to complete dehydration and to infiltrate with paraffin.[2] The schedule given here is for root tips. For objects penetrated less rapidly than root tips the treatment time should be lengthened; for those which are penetrated more rapidly, it may be shortened.

[1]A crystal violet staining schedule for root tips fixed in Craf will appear in a forthcoming issue of this journal.

[2]Larbaud. Nouvelle technique pour les inclusions et les préparations microscopiques des tissus végétaux et animaux. Compt. Rend. Acad. Sci. Paris, 172, 1317–9. 1921.

1. Fixation with Craf:
   SOLUTION A. Chromic anhydride ($Cr O_3$) 1 g., glacial acetic acid 7 cc., distilled water 92 cc.
   SOLUTION B. Neutral formalin (35 to 40% formaldehyde) 30 cc., distilled water 70 cc.
   Mix equal parts of A and B just before using. Fix for 12 to 24 hours.
2. Transfer the fixed material directly from Craf to 75% ethyl alcohol, changing the solution 3 or 4 times at 15 minute intervals.
3. 85% ethyl alcohol, 1 hour.
4. 80% ethyl alcohol 65 cc. and normal butyl alcohol 35 cc., 1 hour.
5. 90% ethyl alcohol 45 cc. and normal butyl alcohol 55 cc., 1 hour.
6. Absolute ethyl alcohol 25 cc. and normal butyl alcohol 75 cc., 1 hour.
7. Normal butyl alcohol, 2 to 3 hours, changing hourly.
8. Infiltrate with paraffin and embed. Infiltration should be gradual. This may be accomplished by adding melted paraffin (m. p. 54 to 55° C.) slowly to the receptacle (preferably a 30 cc. or 50 cc. pyrex beaker) containing the material in butyl alcohol, in such a manner that the paraffin will solidify on top of the liquid. The amount of paraffin added at this time should not exceed one-third the volume of the butyl alcohol covering the material. Place in a paraffin oven at 56° C. and leave several hours, or over night. As the paraffin melts it passes slowly to the bottom of the beaker and gradually infiltrates the material. Displace the butyl alcohol-paraffin mixture with pure liquid paraffin gradually during a period of 3 or 4 hours. After the material has been in pure paraffin 2 or 3 hours it is ready to embed.

In following this schedule, two days are required to complete the dehydrating, infiltrating and embedding processes for root tip material, in contrast to the 9-day period recommended by La Cour[3] for similar material. Ordinarily, dehydration and the initial step in infiltration are completed the day after fixation of the roots, and the remainder of the schedule is finished on the following day.

With this shorter schedule better results actually are obtained than with the longer schedules commonly used, due in part to the elimination of the injurious effect of washing in water after fixation, thru the direct transfer of the material from the fixing fluid to 75% alcohol, and possibly also to the shortening of the period of exposure to the relatively high temperatures in the paraffin oven.

[3]La Cour, L. Improvement in every day technic in plant cytology. J. Royal Micro. Soc., 51, 119–126. 1931. See p. 122.

NOTES ON BELLING'S GREEN-LIGHT METHOD FOR CRITICAL MICROSCOPY (CONTINUED)[1]

HENRY C. WATERMAN, *Washington, D. C.*[2]

(3). ON THE USE OF THE TERM "STENOCHROMATIC" TO DISTINGUISH AND DEFINE THE TRANSMISSION OF CERTAIN FILTERS. The yellow-green and blue-green filters most useful in microscopy transmit a single and rather narrow band (in the visible spectrum). Obviously, such a band is not monochromatic in the strict sense; but it is also quite distinct from the light transmitted by dichroic filters and by filters which do not cut off any part of the visible spectrum completely, or cut off only small portions at one or both ends. To describe such narrow-band filter transmission as "approximately monochromatic," however, is clumsy and not very informing at best; and it may, apparently, actually be misleading.

The writer, therefore, proposes the term "stenochromatic" (στενός, = "narrow"), as being at the same time correctly descriptive, fairly self-evident in meaning, and suggestive of the relation between a "narrow-colored" band and a "single-colored" spectral line; a stenochromatic band being defined as one including a portion of the visible spectrum not wider, in terms of the wave length range covered, than the average width of one spectrum "color," as conventionally recognized, or not more than about 70 mμ of wave length. "Stenochromatic transmission" would then be defined as the transmission of but one such band, with no appreciable transmission in any other part of the visible spectrum.

To define the actual width of any particular "stenochromatic band," the writer suggests the convention, a (c,—%) b mμ, "a" being the lower, and "b" the upper limiting wave length of appreciable transmission, and "c," the wave length of maximal transmission, to be followed within the parentheses by the percentage of transmission at that wave length.

For example, the statement that the combination of Wratten light filters, No. 15 + No. 45, "gives a stenochromatic transmission of approximately 510 (525, 5.4%) 540 mμ," is both concise and definitely informative.

(4). A "STENOCHROMATIC" BLUE-GREEN LIGHT FILTER OF VALUE IN MICROSCOPY. A blue-green filter giving a stenochromatic transmission of about the same width as that given by the yellow-green combination of Wratten filters, No. 15 + No. 45, previously[1] recom-

[1]For the preceding notes of this series see: Waterman, H. C. Notes on Belling's green-light method for critical microscopy. Stain Techn., 9, 21–22. 1934.

[2]No. 2221 Randolph St., Woodridge, N. E., Washington, D. C.

mended can be made by mounting together the Wratten light filter films, No. 47 + No. 61. The transmission of this combination is approximately 490 (500, 2.3%) 520 mμ. As the maximum transmission figure indicates, a rather powerful light is required. The lamp to be described by the writer in a later paper provides sufficient intensity for all ordinary work, however, if the magnification is not carried much beyond the "maximum useful magnification" figure of *working* N.A. multiplied by 1000. The writer uses the No. 47 + No. 61 combination with the optical combination, O. I. objective, N.A. 1.3, × 95, and eyepiece × 15, aplanatic condenser opened to about N.A. 1.25, the total magnification (× 1425) thus being somewhat more than 1110 times the working N.A.; and the illumination is adequate without using the full capacity of the lamp. An intensity sufficient for the binocular instrument can also be obtained.

The effect of the shorter wave lengths transmitted, in still further improving resolution, is often noticeable in comparing this filter with the No. 15 + No. 45 combination.

(5). A Rapid and Optically Satisfactory Method of Mounting Gelatin Film Light Filters. Altho Wratten gelatin film filters can ordinarily be had promptly, *combinations*, mounted in balsam, have to be made to order, which necessitates much delay.[3]

Two-inch squares of the films required for a combination filter may be mounted easily and quickly between 2⅛ in. squares of ⅛ in. plate glass by putting one or two large drops of cedar-wood immersion oil on one of the carefully cleaned glasses, adding one of the films and gently pressing out any air bubbles which may be formed, then adding more oil and the second film, and, after again removing bubbles, adding a third layer of the immersion oil and the second glass. After the excess oil has been wiped off, the edges of the mount are thoroly freed from oily film (to insure the adhesion of a water-soluble gum) by means of a cloth dampened with benzol or xylol and the mount is bound with lantern slide binding strip. Care must be taken that the binding strip is not moistened more than is necessary, especially if glasses no larger than the squares of film are used, since a dampening of the edges of the gelatin causes a swelling which both injures the film and opens the mount, admitting air.

Optically, the immersion oil is, of course, quite equal to Canada balsam. It has two advantages over balsam, moreover, as it does not require a volatile solvent and consequently does not contract on hardening, by loss of solvent, and requires no warming (rather risky in the case of gelatin films) to keep it fluid enough for the convenient removal of bubbles formed in the mounting process.

A very slight oozing of the oil, usually at one of the corners of the

[3]Wratten light filters. The Eastman Kodak Co., Rochester, N. Y. 1932. See p. 4.

mount, ceases entirely after a few days. If small air bubbles do appear, they are easily removed by taking off a part of the binding from the nearest edge, squeezing out the air and adding a small drop of immersion oil before releasing the pressure, and then cleaning and rebinding the opened edge as in the original mounting. The writer, however, has had to make this repair but once.

(6). THE FRONT-SILVERED SUBSTAGE MIRROR IN A BETTER AND MORE PERMANENT FORM. The writer has already called attention to the optical effectiveness of a front-silvered mirror in avoiding multiple images of the light-source (see photomicrographs, Fig. 1), and to the possibility of using it in place of the more bulky and expensive reflecting prism used by Belling[4] as a substitute for the common plane

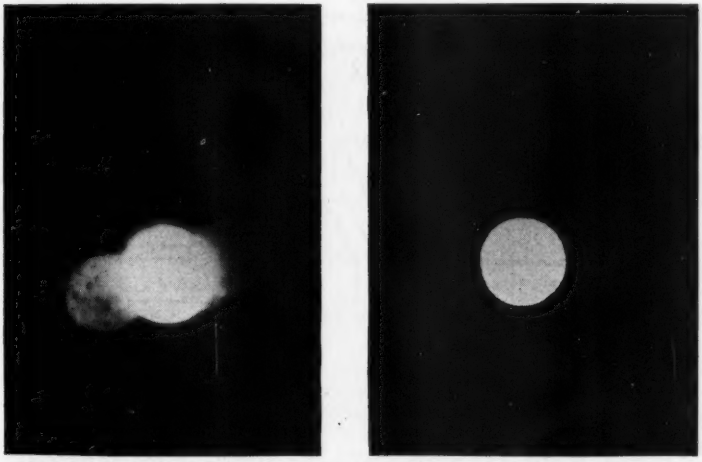


Fig. 1. EFFECT OF MICROSCOPE MIRROR ON IMAGES OF SMALL DIAPHRAGM-OPENING, AS SEEN IN THE MICROSCOPE

A. Ordinary microscope mirror, silvered on the back

B. *Front*-silvered ("first-surface") mirror. A reflecting prism gives the same result, except for a slightly greater loss of light.

mirror silvered on the back. In the form in which the writer first suggested this mirror (see footnote 1), the fully exposed silver made the device more or less impermanent, the silver slowly tarnishing until it had to be replaced. A much more permanent form of the front-silvered mirror is available, however, in the *lacquered* "first-surface" mirror used in some makes of camera lucida, in vertical projection lanterns, etc. The lacquer is so thin (and possibly of so low a refractive index) that its optical effect is not detectable; but this film apparently prevents tarnishing for a number of years in an ordinary

[4]Belling, J. The Use of the Microscope. McGraw-Hill, New York. 1930.

atmosphere. Moreover, the silvering is heavier and better polished than that made available by the "make-shift" method first suggested.

In the form just described the "first-surface" mirror would seem to be optically a little better than the prism, in that the prism is subject to a light loss of about 4% at the surface normal to the illuminating beam, and to a further loss, by absorption in the glass itself, of probably as much as 3 or 4% more.

The holder previously suggested for the improvised form of the front-silvered mirror serves equally well for the lacquered variety. The dimensions obviously need not be rigidly specified, but the following construction has proved satisfactory: Bottom plate of $\frac{1}{16}$ in. brass, $2\frac{1}{8} \times 1\frac{3}{4}$ in.; sides, a strip of $\frac{1}{32}$ in. brass, $\frac{5}{16}$ in. wide, bent to fit smoothly and tightly around the bottom plate, and soldered with the blowpipe; slip-in frame, 4 pieces of $\frac{1}{8}$ in. square, brass rod laid together and soldered, in one heating, with the blowpipe. The pieces are conveniently held in place for the soldering with pins on a piece

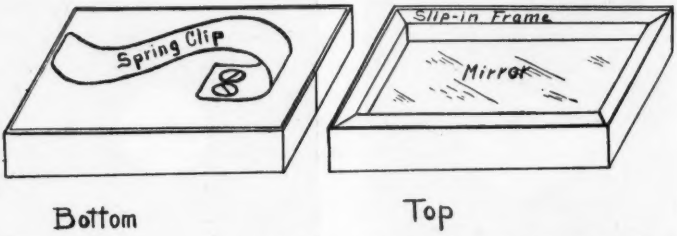


Fig. 2. Holder for Front-silvered Mirror, with Spring Clip for Attaching Holder to Ordinary Microscope Mirror

of asbestos board. This frame holds the mirror in place, as indicated in Fig. 2. The writer places a piece of felt on the floor of the box part so that the mirror may be pressed down firmly with the frame without danger of cracking it. A piece of thin chamois leather is folded over the regular mirror of the microscope to protect it from scratching when the holder is slipped on.

In the holder described, the front-silvered mirror can be removed when not in use, and given the much needed protection from mechanical injury by keeping it face down in a suitable box. The lacquered surface *must not be touched with the fingers*, since the marks can hardly be removed without injury to both lacquer and silver.

A piece of lacquered "first-surface" mirror of the size indicated, and made on $\frac{1}{8}$ in. plate glass, can be secured at a very reasonable price, from the Bausch and Lomb Optical Co., Rochester, N. Y., if one can wait until they are ready to prepare a regular batch of such work.

A PERMANENT ROOT TIP SMEAR METHOD

HARRY E. WARMKE, *Stanford University, California*

ABSTRACT.—**Kill root tips in 1 part glacial acetic acid to 3 parts absolute alcohol for 12 or more hours. Remove from killing fluid and place for 5 to 10 minutes in a solution consisting of 1 part 95% alcohol to 1 part concentrated HCl. Transfer to Carnoy's fluid for 5 minutes or longer. Cut a small piece (0.5 mm. or less) off the tip of the root and place on a clean slide in a small drop of iron-aceto-carmin stain. Press directly on the piece of root with a small flat scalpel; the cells will now separate and float free in the stain. Place cover slip over the drop of stain and apply gentle pressure. Heat carefully by passing the slide 3 or 4 times thru the flame of an alcohol lamp. Seal with heated mixture of 1 part Parowax to 1 part gum mastic. Make permanent by the McClintock permanent method.**

The permanent root tip smear method herein described was devised to make possible the study of chromosome numbers and morphology in the genus *Trillium*, where the chromosomes are so large and closely packed that paraffin section technic is totally inadequate. It has also been used successfully on other plant genera, including *Allium*, *Crocus*, and *Lathyrus*.

Kill and fix root tips for 12 hours or longer in 1 part glacial acetic acid to 3 parts absolute alcohol. The root tips will keep two or three weeks in this solution; for longer storage they should be transferred to 70% alcohol.

After killing, place roots in a solution consisting of 1 part 95% alcohol to 1 part concentrated HCl for 5 to 10 minutes. This mixture dissolves the pectic substances of the middle lamella and thus allows the individual cells to separate when pressure is applied. It may be varied considerably; a solution of 3 parts HCl and 7 parts 95% alcohol has been used, but the time must be prolonged. In spite of its apparent severity, it has been found that the 1:1 mixture does not distort the chromosomes. Trabants, constrictions, internal structure, and other cytological details are preserved. In fact, by this new smear technic, the author discovered a trabant that he did not know was present on a species that he had studied extensively by the section method.

After this treatment transfer root tips to Carnoy's fluid (with chloroform) for 5 or more minutes; this hardens the tissue again after the HCl treatment, which more or less softens it. Remove from Carnoy's fluid, cut a small piece off the tip of the root and place it on a

clean slide in a small drop of iron-aceto-carmin stain. Difficulty will be experienced later in flattening the cells sufficiently if too large a piece is used; the embryonic region of an onion or *Trillium* root should be divided into 3 or 4 pieces. The pieces are cut off as needed, each piece going to make one slide; thus 3 or 4 slides may be made from a single good root tip. The drop of iron-aceto-carmin also should be small. If too much stain is used, it will flow out from under the cover slip and carry many cells with it. From here on the method follows the regular iron-aceto-carmin method[1] quite closely.

With a small flat scalpel press directly on the piece of root tip several times until the cells have all separated from one another in the stain. Now place a cover slip over the drop of stain and suspended cells. Small cover slips have been found superior to large ones; ½ inch squares are a convenient size.

Next apply gentle pressure to the top of the cover slip with a flat, blunt instrument such as the end of an unsharpened pencil. Sufficient pressure should be applied to flatten the cells but not to crush them. The slide is now heated by passing it 3 or 4 times thru the flame of an alcohol lamp or over an electric plate. Care should be taken not to boil the solution under the cover slip.

The slide may be preserved temporarily by heating together equal parts of Parowax and gum mastic and applying the mixture, while hot, around the edge of the cover slip with a match or a splinter of wood. In this condition the slides will keep for a week or ten days and may be examined critically; the good ones may be saved and the unsatisfactory ones discarded.

The good slides are preserved permanently by the McClintock[2] permanent method. Briefly, as much of the seal as possible is removed with the aid of a razor blade, without moving the cover slip. The slide, with cover slip in place, is then submerged in 10% acetic acid. One corner of the cover is carefully lifted by means of a small sharp scalpel and the cover slip allowed to float free in the liquid. Both slide and cover are run up thru the following series: 1:1 absolute alcohol and glacial acetic acid; 3:1 alcohol and acetic acid; 9:1 alcohol and acetic acid; 100% alcohol; 7:3 alcohol and xylene. From this last stage the slide and cover slip are recombined with balsam; this should be done as rapidly as possible to avoid absorption of too much moisture from the air. It is preferable to run on up to 100% xylene before

[1]Belling, J. The iron-aceto-carmin method of fixing and staining chromosomes. Biol. Bull., **50**, 160–2. 1926.

Sax, Karl. The smear technic in plant cytology Stain Techn., **6**, 117–22. 1931.

[2]McClintock, B. A method for making aceto-carmin smears permanent. Stain Techn., **4**, 53–6. 1929.

mounting if the pure xylene does not cause shrinkage or discoloration.

The author has been using this method for several months and has found it entirely practical. He has been able to photograph root tip figures of all Pacific Coast *Trilliums*, so that the diploid chromosome numbers are countable from the pictures. (See text figures 1, 2, and 3.) Class preparations of onion root tips have been made that clearly show the 16 metaphase chromosomes spread out in one plane.

Fig. 1

Fig. 2

Fig 3

Photomicrographs of permanent root tip smear preparations to illustrate possibilities of the method. All figures are flattened. Fig. 1: *Trillium chloropetalum*, showing 10 chromosomes in each anaphase group; Fig. 2: *Trillium ovatum*, showing 10 metaphase chromosomes; Fig. 3: *Trillium grandiflorum*—anaphase figure showing 10 chromosomes directed toward each pole.

The same method may also be used to study the first division in the microspore.[3] The HCl causes the heavy exine wall to split and fall away, like the shell of a nut, leaving the naked protoplast adhering to the slide.

Of course, as with all smear methods, care must be exercised to select figures that show a minimum of distortion; nevertheless, this method has many real advantages, among which are rapidity, permanence, the possibility of flattening figures so chromosomes lie in one plane, and the high percentage of good preparations.

[3]Warmke, H. E. and Johansen, D. A. The chromosomes of the Californian Liliaceae II. Am. J. Bot. (in press).

A MODIFICATION OF THE CRESYL VIOLET TECHNIC FOR STAINING NERVE CELLS

GRACE TRESS AND MILDRED TRESS, *Institute of Neurology, Northwestern University Medical School, Chicago, Ill.*

Sections stained with cresyl violet may be differentiated in very weak acid alcohol (100 cc. of 95% alcohol and two drops of 1% hydrochloric acid), or in absolute alcohol to each 100 cc. of which two drops of neutral Canada balsam have been added. The first method does not give very satisfactory results; the second is slow and expensive. The method which is outlined in this paper gives as good cell differentiation as the second method just mentioned, and is more rapid and less expensive. It is applicable to material embedded in nitrocellulose, celloidin, or paraffin, and to sections ranging from 10 to 50μ in thickness. It removes any mucin bodies that may be present, and the staining and differentiating can be rapidly accomplished. It is especially to be recommended when relatively thick sections are used, as is often desirable in neurological work.

Alternate sections may be stained by Weil's[1] method for myelin sheaths and by cresyl violet for cells, a single series thus serving for the study of cell groups as well as fiber distribution.

The tissue, fixed in formaldehyde, U. S. P. 1:10, is embedded, preferably in nitrocellulose,[2] cut into sections of the desired thickness and stained by the following method:

1. Wash sections in distilled water.
2. Stain in the following solution for 30 minutes at 50° C.:

Cresyl violet	0.5	grams
Distilled water	100	cc.
Glacial acetic acid (diluted 1:10)	4	drops

Filter the stain before using. The staining solution can be used repeatedly; the time of staining should be increased when the solution becomes weak.

3. Wash in distilled water.
4. Differentiate in 70% alcohol until most of the stain is removed from the nitrocellulose.
5. Place the sections in the following solution for 2 to 5 minutes:

Chloroform	60 cc.
Absolute alcohol	10 cc.
Ether	10 cc.

[1]Weil, A. Rapid method for staining myelin sheaths. Arch. Neurol. and Psych., 20, 392. 1928.

[2]Davenport, H. A. and Swank, R. L. Embedding with low viscosity nitrocellulose. Stain Techn., 9, 137–9. 1934.

Be sure that the sections are kept completely submerged. Practically no differentiation of the cells takes place, but the stain is removed from the background.

6. Differentiate until the cells are slightly darker than desired in 100 cc. of 95% alcohol to which have been added 4 drops of 1% hydrochloric acid. This acidity is optimum for most sections, but sometimes it is necessary to either increase or decrease the concentration.
7. Neutralize the sections in 95% alcohol which has been alkalinized by the addition of a small amount of sodium bicarbonate.
8. Wash the sections in 95% alcohol to remove any remaining sodium bicarbonate.
9. Complete the dehydration in 2 changes of normal butyl alcohol. Absolute alcohol can be substituted, if desired, for the butyl alcohol if the sections are either celloidin or paraffin embedded.
10. Clear in 4 changes of xylol.

Cresyl violet (cresylechtviolett) from Dr. Grübler and Company has been used exclusively in our laboratory. A mold will grow in the cresyl violet solution; filtering before use will remove this and the solution can be used as long as it is concentrated enough to give a satisfactory stain. The stained sections will fade if exposed to direct sunlight or heat.

NOTES ON TECHNIC

A NEW STAINING TECHNIC FOR DESMIDS.—Any desmid-staining process should have the following characteristics: (1) It should be permanent: (2) it should stain the important parts of the cell (this includes chloroplasts, pyrenoids, etc.); (3) it should be simple and convenient. The following procedure has been employed by the writer for a period of 2 years on various genera of desmids.

Desmids fix well in 2 to 3% formaldehyde or 2% osmic acid. If the former is used the entire culture may be placed in the killing agent. If the latter is used a drop of the desmid culture may be simply inverted over the bottle of acid. Formaldehyde seems to be advantageous from the standpoint of field collecting. A few drops of acetic acid will counteract any tendency to shrink in the formaldehyde.

After killing and fixing, the process is essentially one of decantation. The killing medium is decanted and the various grades of alcohol added and in turn decanted. The decantation process may be simplified and speeded up by use of a hand centrifuge. The alcohols should be in grades of 2½%, 5%, 10%, 15%, etc., to 95%. At this stage the culture should be left at least over night in an alcoholic solution of light green (1 g. to 100 cc. of 85% alcohol). Fast green stains well but does not give a green color as close to the normal desmid green as that given by light green. The period of staining varies with the type of desmid. Periods of 48 hours or more are desirable for some species of *Micrasterias* and *Staurastrum*. Nuclei very often take the stain after prolonged periods in the solution.

After staining, the gross culture should be washed in 95% and absolute alcohol for periods from 15 to 60 minutes, the length of time depending on the strength of the stain. It is helpful to watch the process in depression slides under compound binoculars. After washing in absolute alcohol the material should be cleared in xylene in series 2½%, 5%, 15%, etc.

For isolation the individual plant may be removed from the clearing solution by means of a small bit of balsam stuck to the end of a very fine needle or drawn-out glass tubing.

The advantages of the foregoing process are several. First, the desired plant need not be isolated until staining is complete. The process is simple and permanent. Second, the desired parts of the plant are stained, namely the chloroplasts, pyrenoids, nuclei, etc. A green color that is almost that of the original is restored. Third, handling, especially of individuals, is reduced to a minimum.

Summary: Fix in 2 to 3% formaldehyde with a few drops of acetic acid added. Run up by decantation thru the various grades of alcohol of the multiples of 5% series. Ten to 15 minutes will suffice for each grade. Stain for 12 to 48 hours in light green SF yellowish or fast green FCF; 1 g. of stain to 100 cc. of 95% alcohol. Wash in 95% and absolute alcohol for a period of 10 to 15 minutes in each grade. Clear in the xylene series; mount in balsam.—GORDON D. ALCORN, Dept. of Botany, Univ. of Wash., Seattle, Wash.

THE PRESERVATION OF MOLDS FOR CLASS USE.—In an attempt to resolve the *d* and *l* forms of secondary butyl alcohol (CH_3CH_2-$CHOHCH_3$) by inoculation with various molds, it was found that the alcohol was immediately toxic to the organisms used. In very low concentrations (½ to 1%) growth of the molds was stopped, and upon transferrence of both hyphae and spores to culture media no growth was obtained, the alcohol proving lethal to them.

This observation has suggested that the alcohol may be used for the preservation of samples for class use, since it makes unnecessary the usual technic of starting cultures several weeks beforehand, and then retarding their growth by refrigeration. When the organisms have reached the desired stage of development, they are transferred from the culture media into dilute aqueous solutions of secondary butyl alcohol. The samples then may be kept in stoppered vials for several months until required for class work.

Plasmolysis is slight or entirely lacking in some of the common forms (*Saprolegnia*, *Mucor*, *Aspergillus*, etc.) even after six months in a 2% solution. Such a solution (2 cc. of the alcohol in 100 cc. of water) has a calculated osmotic pressure of about 6 atmospheres, but since the osmotic pressure of a mold is dependent upon that of its medium, no definite concentrations for specific organisms can be recommended. By trial of various concentrations in the range suggested, however, one may easily be found which will preserve the mold without plasmolysis in the identical condition prevailing at the time of immersion.

The use of secondary butyl alcohol for the lengthy preservation of fleshy fungi and higher plants in herbaria jars is also indicated, but investigations have not proceeded for a sufficient length of time to warrant statement of its full value.—R. E. OLTMAN, Dept. of Botany, Univ. of Minn., Minneapolis.

LABORATORY HINTS FROM THE LITERATURE

Under this heading are given interesting new methods in microscopy that have been noticed in other publications. The readers' assistance is urged in calling the editor's attention to any outstanding methods in this field that are overlooked.

BIER, OTTO and ROCHA E SILVA, M. Action des solutions concentrées de NaCl sur l'hémolyse et la fixation des érythrocytes par l'éosine irradiée. Compt. Rend Soc. Biol. (Paris), **118**, 914–5. 1935.

Sun-irradiated eosin fixes erythrocytes at concentrations above 0.25%; hemolyzes them at dilutions higher than 0.032%. At pH 6.6, M/5 (or stronger) NaCl solutions protect the cells from hemolysis in a 0.05% eosin solution. Fixation is only partial in presence of 0.5% eosin and M/7 NaCl.

BIER, OTTO and ROCHA E SILVA, M. Action du KCN sur l'hémolyse photodynamique. Compt. Rend. Soc. Biol. (Paris), **118**, 911–3. 1935.

If a suspension of red blood cells in saline is mixed in a test tube with eosin, exposure of the mixture to sun irradiation may cause either hemolysis or fixation of the cells, depending upon the concentration of the dye.

This photodynamic hemolysis can be compared to some oxidations which require the presence of light as the source of energy, of atmospheric O_2, and of a fluorescent dye as a photosensitizer. In this case it is assumed that a peroxide is formed, whose oxygen is used in its active form to bring about oxidation of the cell materials. Considering, therefore, the photodynamic hemolysis as a dehydrogenation process within the system erythrocyte-eosin, with H_2O_2 being formed, it is found that KCN ions, from concentration M/32 to M/128 at pH 6.6, intensify considerably the hemolysing and fixing action of eosin exposed to sunlight for one hour.

DUFRENOY, J. and REED, H. S. The choice of killing fluids appropriate for cytological research. Science, **81**, 273–4. 1935.

The study of cell morphology rests upon the results of proper killing and staining. The killing fluid should penetrate rapidly and preserve all existing conditions, including apparent homogeneity of the cytoplasm, clear-cut appearance of mitochondria and plastids, outlines of vacuoles and metabolic products. Meves' killing fluid stains the lipids and precipitates the phenolic compounds, but takes one week to penetrate and may produce artifacts. Nemec's fluid is quick and safe. For best results it should be followed by a 3% $K_2Cr_2O_7$ treatment for at least one week to mordant mitochondria and other constituents.

GERARD, P. Sur l'emploi du noir soudane B. pour reconnaître les inclusions de vaseline liquide. Bull. d'Histol. Appl., **12**, 92–3. 1935.

For the diagnosis of intrusion into tissues of foreign organic substances, like camphorated liquid vaseline, the usual fat stains cannot afford any differentiation. Blue B. Z. L., Sudan red B, and "bleu cibacète B" are similarly unsatisfactory. Sudan black B in 15 minutes will stain fatty materials a deep blue-black, the vaseline a light violet. These dyes have been introduced into histology by Lison; see paper, abstracted below, by Lison and Dagnelie (Bull. d'Histol. Appl., **12**, 85).

GIEMSA, G. Geschichte, Theorie und Weiterentwicklung der Romanowsky-Färbung. Med. Welt, No. 41, 1432. 1934.

This paper is a survey of the Romanowsky blood stain history, from the uncertain years of its first appearance to the time of more careful study as performed by Nocht, Giemsa, and Michaelis. The usual technic has undergone several minor changes and modifications, moreover; as, for instance, in the "quick stain," in Neri's improvement, and in Regendanz and Reichenow's applications to sectioned materials.

GUARDABASSI, M. Sur la structure des corps de Negri dans les photomicrographies a l'infrarouge. Compt. Rend. Soc. Biol. (Paris), **118,** 559–61. 1935.

The use of infra-red rays in cytology offers distinct advantages. The finest structures are outlined in a remarkably clear manner, without requiring endangering handling or manipulations of materials. Sections of brain of a rabid dog fixed in alcohol, sensitized with rubrocyanine *Agfa*, and examined under the microscope, show the Negri bodies to have assumed a deeper green-bluish tinge than the surrounding cytoplasm; the edges are well defined, the inside structure less so. Upon infra-red micrographic examination, however, the Negri body reveals a granular structure with threads connecting the granules which are also present in the so-called capsule.

HORGAN, E. S. The failure of brilliant green and telluric acid as selective agents for the isolation of *Bacillus typhosus* from faeces. J. Hyg., **35,** 38–45. 1935.

Attempts in the Sudan at isolation of the typhoid organism from feces by means of the McConkey medium (satisfactory for dysentery bacilli) were a failure. Even feces inoculated with the organism, suspended and incubated in brilliant green-telluric acid peptone water, failed to show growth. Dilutions of the dye ranged from 1/125,000 to 1/500,000; telluric acid concentration 1/12,500; pH 6.5. Pre-war and after-the-war Grübler dye samples were found to be equally unsatisfactory. Author hints at the fallacy of applying standard methods, worked out in temperate climates, to tropical conditions.

HUREL-PY, G. De la réaction de Feulgen appliquée aux chondriosomes. Compt. Rend. Soc. Biol., **118,** 1168–71. 1935.

Opinions as to stainability of chondriosomes and plastids by means of the Feulgen method are discordant. The author mixes her material in "a concentrated solution of sublimate" or in 95% alcohol for 24 hours, followed by a 24-hour wash in running water or renewed alcohol. Hydrolysis of 4 to 15 minutes, followed by 1 to 2 hours staining of epidermic cells of the perianth of a tulip, are without effect on plastids and chondriosomes. Trials with *Allium cepa, Hyacinthus orientalis, Elodea canadensis, Rosa canina*, fungi and algae are also negative.

KLINE, E. K. Toxicity of brilliant green for certain bacteria. Am. J. Pub. Health, **25,** 314–8. 1935.

This paper reports a study of the toxic effect of various brilliant green dyes incorporated in a culture medium for differential bacterial growth. This work, by the referee of the Section on Diagnostic Methods and Procedures of the Standard Methods Committee, is a search for methods on the standardization of bacteriostatic properties of dyes and has been summarized in an editorial elsewhere in this journal (Stain Techn., **10,** 2, 1935).

LENDRUM, A. C. Celestin blue as a nuclear stain. J. Path. & Bact., **40,** 415–6. 1935.

This nuclear stain, as suggested by Proescher and Arkush, is made up by boiling 3 minutes 0.5% celestin blue, C. I. 900 (Revector Co., London) in a 5% solution of iron alum in distilled water, and filtering after cooling. By adding 14% of glycerol and 2% of concentrated H_2SO_4 the stain solution holds stable for several months. Good nuclear definition is obtained in 5 to 15 minutes; occasional cytoplasmic staining is removed by acid (HCl) alcohol. As counterstain 1% chromotrope 2R, C. I. 29 (Revector Co., London) in absolute alcohol is suggested. Celestin blue can be used in the original Van Gieson method, and in Masson's or Benda's modifications. A method is evolved to study breast carcinoma and skin lesions: fix in Zenker or Bouin's fluid; stain the elastin by Ruben Duval's method and bring to water; stain nuclei 15 minutes with celestin blue and wash 20 minutes in water; stain muscle and epidermis 2 minutes with eosin (2 pts. 1% aqueous eosin plus 1 pt. 0.5% aqueous gallic acid). Decolorize the collagen in water or 30% alcohol and then 2 minutes in 1% aqueous phosphomolybdic acid. Wash; stain collagen with Masson's light green solution; rinse in 1% aqueous acid; dehydrate rapidly. Results: elastin, bright brown; nuclei, blue purple; muscle and epidermis, red; and collagen, green.

LENDRUM, A. C. On the cutting of "difficult" tissues. J. Path. & Bact., **40**, 416–7. 1935.

For rapid diagnosis the following phenol-containing modification of Carnoy's fluid is suggested: 75 parts absolute alcohol, 10 parts water, 10 parts chloroform, 4 parts phenol crystals; 3% of glacial acetic acid may be added just before use. Dehydrate with 4% phenol in absolute alcohol, clear in benzol. The soaking in water of paraffin blocks of human pathological material does not prove helpful. Formalin or Zenker fixed tissues are transferred after washing to 4% phenol for 24 to 72 hours and then dehydrated; Bouin's material is dehydrated 24 to 72 hours with 4% phenol in absolute alcohol, followed by straight absolute alcohol. Insertion of a "phenol stage" is found to be most effective just before dehydration.

LIPPERT, K. M. The photodynamic effect of methylene blue on tetanus toxin. J. Immunol., **28**, 193–203. 1935.

Toxicity of tetanus toxin in a filtered broth culture is decreased by the photodynamic action of methylene blue (Grübler). The toxin was mixed with a 0.9% NaCl solution and varying amounts of the dye. The mixture was exposed for 1 hour to a 187 candle power light bulb. The most effective concentration of the dye was found to be 0.005 mg. per cc. of the mixture. Inoculation of the dye subsequent to toxin injection into rabbits did not neutralize the latter.

LISON, L. and DAGNELIE, J. Méthodes nouvelles de coloration de la Myéline. Bull. d'Histol. Appl., **12**, 85–91. 1935.

(1) Staining of myelin proper: This is a method of *direct* staining of the myelin by means of a fat-soluble dye, assuming the myelin as being made up of lipids. Sudan III alone or with phenol, and "Scharlach" (Sudan IV?) give only faint coloring. The dye Sudan black B (Sudanschwarz B, I. G. F.) is used as follows: Fix at least 3 days in neutral 10% formol. Rinse in distilled water, freeze and cut (generally 30 μ). Soak 1 minute in 50%, then 1 minute in 70% alcohol. Stain from 15 minutes to several hours in a filtered saturated solution of Sudan black B in 70% alcohol. The dyestuff solution should not be more than 6 months old. Rinse a few minutes in 50% alcohol, and in distilled water; apply 10 to 12 minutes, if wanted, carmalun diluted to ⅓ or ¼, mount in glycerin-gelatin or de Heringa and ten Berge's medium. Myelinous fibers appear black on a clear or slightly greenish background. Only other fatty structures take the same stain. The granular bodies are opaque black and the chromolipoid pigments generally green blackish.

(2) Staining of degenerative myelin: The dye is the Blue B. Z. L. (Ciba) used in strongly diluted alcohol solution to spare fatty materials from dissolving; it stains sky blue and does not overstain. The technic is exactly as the above except for 3 rinses of 1 minute each in 50% alcohol prescribed before applying the stain made up in 40% alcohol. Rinse directly in distilled water.

MEYER, K. F. and JOHNSTONE, H. G. Laboratory diagnosis of amebiasis. Am. J. Pub. Health, **25**, 405–14. 1935.

Of seven essential steps for a correct diagnosis of *Entameba histolytica*, discussed by the writers, two are of special interest: (1) Preparation of stool specimens for examination; and (2) Microscopic examination of stained preparation. Only exceptionally should fresh unstained stool preparations be employed. For preliminary observation of fresh smears use either Kofoid's modification of Donaldson's iodine-eosin stain, (see Kofoid, Kornhauser and Swezy. Arch. Int. Med., **24**, 43:35–50, 1919) or better, MacDaniels' methylene-blue-methyl-alcohol stain (see Stain Techn., **9**, 110). Trophozoites and cysts are easily detected under low magnification as clear refractive bodies in the dark blue field. A definite diagnosis is made on permanent smears prepared by hot or cold fixation of wet smears in Schaudinn's fluid followed by a mordant of iron alum and stained in hematoxylin. (Consult the author for further information). The microscopic scrutiny must consider: the type of cellulor exudate, the various protozoa of the intestinal human tract, and the plant and animal cells of the food detritus present.

ONO, K. Färbung der mit Thrombocyten beladenen Rekurrensspirochäten. Acta dermatol. (Kyoto), **22**, 113–8. 1933. (Abs. in Zentbl. Bakt., I Ref., **116**, 488. 1935.)

In blood smears it was possible to show the spirochetes of relapsing fever by a 15-minute treatment with 10% formalin, or 3% H_2O_2, or 1% $KMnO_4$. Sixty-three dyes were found suitable for this stain, used in 1% aqueous solution. A few of them were: ethyl violet, crystal violet, dahlia, fuchsin, gentian violet and Victoria blue.

ONO, K. Studien über die Färbung der Rekurrensspirochäten, insbesondere über den Zusammenhang der Färbung mit den Vorbehandlungen. Acta dermatol. (Kyoto), **23**, 1–14, 39–55. 1934. (Abs. in Zentbl. Bakt., I Ref., **116**, 486–7. 1935.)

I. Einfluss der Formalinfixierung auf die Färbbarkeit der Rekurrensspirochäten: Dry blood smears from mice infected with Manchurian relapsing fever were treated 15 minutes with 10% formalin, thoroly washed, and stained immediately. As controls other blood smears were fixed in alcohol-ether before staining. For this, 453 various organic dyestuffs were tested out in 1% solution in water and for 15 minutes. Formalin fixation caused spirochetes and leucocytes (especially thin neuclei) to take on the colors more strongly, while the erythrocytes were more weakly stained. The blood plasma remained unstained. Following fixation with alcohol-ether, 63 dyestuffs failed to stain the spirochetes and leucocytes and 46 stained them only slightly; a satisfactory stain was secured in these cases after formalin fixation. Particularly good results were gained after formalin by the use of 78 of the dyestuffs, among which were ethyl violet VA, various crystal violets, fuchsin G, dahlia H, sky blue VR, methyl violet, rose bengal, safranin, and Victoria blue.

II. Einfluss der Länge der Aufbewahrungszeit der Strichpräparate auf die Färbbarkeit der Rekurrensspirochäten: If blood smears are kept 10 days before being fixed in alcohol-ether and stained, the staining of the blood plasma appears to be much stronger, while the leucocytes are not so brilliant. There are dyestuffs that cannot stain the spirochetes in a freshly made smear, but can bring them forth, negatively, in a preparation held for a time. Other dyestuffs may stain them fresh, but show them negatively in a preserved preparation. The influence of the length of preservation on the stainability of the relapsing fever spirochetes is independent of the chemical make-up of the dyestuff.

III. Einfluss der Vorbehandlungen mit verschiedenen Fixierungs- und Beizmitteln auf die Färbbarkeit der Rekurrensspirochäten: A difference in the influence of various fixatives and mordants on the stainability of spirochetes and blood elements was to be noticed only on fresh smears. Materials used were: alcohol-ether, alum, acid ammonium molybdate, $K_2Cr_2O_7$, Müller's solution, Orth's mixture, and particularly formalin, H_2O_2, and $KMnO_4$. Treatment with chromic acid permitted a clear negative picture of the spirochetes by means of the acid dyes. Of the many dyestuffs successfully used as spirochete stains the following shall be mentioned: crystal violet P, navy blue D conc., methyl violet *non plus ultra*, red violet 4R, and Victoria blue 4R hc.

IV. Einfluss der Vorbehandlung mit Wasserstoffsuperoxydlösung auf die Färbbarkeit der Rekurrensspirochäten: A 15-minute treatment of blood smears with 3% H_2O_2, as compared with the alcohol-ether fixation, resulted with all dyes in a stronger staining of the relapsing fever spirochetes and leucocytes, weaker staining of the erythrocytes, while the blood plasma remained unstained. After alcohol-ether, 60 of the 282 dyestuffs tested did not stain the spirochetes, and 88 stained them only slightly; after H_2O_2 they stained organisms and leucocytes equally well. The majority of the 66 most suitable dyes belong to the group of the triphenylmethane dyes, as for example, acid violet CBB, crystal violet, dahlia, and fuchsin.

PACHECO, G. and RODRIGUES, C. Biologie des bactéries du groupe *pullorum-gallinarum*. Action sur les milieux au lait et sur le rouge neutre. Compt. Rend. Soc. Biol. (Paris), **118**, 1019–22. 1935.

Neutral red added to culture media may represent a mean of differentiation between species of microörganisms. It acts in two ways: by the changing of the color (from purple-red to canary yellow) when the reaction changes from acid to alkaline, and by the development of a green fluorescence due to reduction. Thirty-eight strains of organisms of the *pullorum-gallinarum* type show that glucose is not necessary for the production of fluorescence; anaerobiosis favors it.